STERLING™

national diagnostics

Rapid Silver Stain

- **Fast - fix, wash, and stain in one hour**
- **Easy - reduces prep time, fewer reagents to mix, and one staining solution**
- **Sensitive - detects sub-nanogram levels of DNA and 5 ng of Protein**

National Diagnostics' STERLING Silver Staining System offers high sensitivity silver staining (nanogram detection of protein and DNA) faster and more conveniently than any available kit. The unique chemistry of STERLING allows the staining to be carried out in a single solution, whereas standard silver stain procedures require nine or more solutions and washes after fixation. Simply fix a polyacrylamide gel using our unique fixative, wash, and place the gel in the STERLING Staining Solution. Bands will appear in 5-10 minutes. It's that simple.

DNA appears as dark brown/black bands on a clear background. Levels as low as 0.1 ng/band can be detected. Proteins, at levels as low as 5 ng/band, appear as black/brown bands. And the entire procedure is completed in one (1) hour.

The STERLING Silver Staining System is supplied as a kit, containing 450ml of Stain Reagent A Concentrate, 60 grams of Stain Reagent B (powder) and 100ml of Fixative Concentrate. The kit contains sufficient material to stain 18 mini-gels.

Method of Use:

- For mini-gels (10X7cm), use 100ml of each solution.
- For larger gels, increase STERLING volumes appropriately to immerse gel to depth of ~1cm.
- Wash mini-gels in 200ml volumes of water.
- Agitate continuously during all steps.
- Glassware must be clean, and the water should be distilled or high-quality deionized.

EASY AS 1-2-3

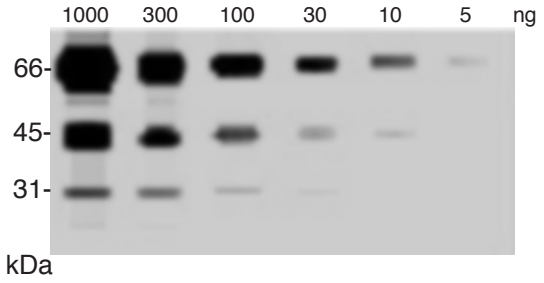
1. **FIX** gel for at least 25 minutes in 100ml of the standard mixture of 5:5:1 Methanol:Water:Acetic Acid.

   Decant fixative, then add reconstituted STERLING Fixative (45ml water, 50ml methanol, 5ml STERLING Fixative Concentrate) and fix for an additional 5 minutes (See Tips and Hints, Fixing Section).

2. **WASH** gel twice for 15 minutes in deionized water. Addition of 0.1% non-ionic surfactant will aid in submerging the gel.
   - While gel is washing, prepare Staining Solution as directed. Do not combine the two component solutions until just prior to use.
3. **STAIN:** Decant wash solution and immerse gel in combined staining solution.

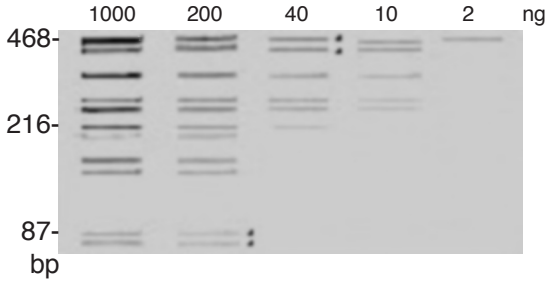
Bands will begin to appear in 5-10 minutes. When desired intensity is achieved, stop development by immersing the gel in a 5% acetic acid solution.

Proteins Visualized by Silver Staining



12% ProtoGel, 8cmX7cmX1mm Mini-Gel. Electrophoresis @ 120V for 1.5 hours. Serial dilutions of protein standards BSA, ovalbumin, and carbonic anhydrase. Gel was fixed and stained following standard STERLING protocol.

DNA Visualized by Silver Staining



6% AccuGel 19:1 in TBE. Electrophoresis @ 120V for one (1) hour. Serial dilutions of Lambda DNA digested with PstI.
* Bands with ~ 0.5ng DNA.

Preparation of Staining Solution:

1. Dilute 25ml Reagent A with 25ml of water.
2. Dissolve 2.8 grams of Reagent B in 50ml of water. Stir until **completely** dissolved (approx. 5-10 minutes).
3. Immediately before use, pour (1) into (2) **with stirring**, and pour over gel. The combined solution has a useful life of ~20 minutes.

TIPS AND HINTS:

• Glassware:

All glassware used in the staining procedure must be scrupulously clean. Wash in soap and water, rinse in high-quality deionized water, wash in Nitric or Chromic Acid and finally rinse with deionized or distilled water for best results. Residue on the surface of the staining tray will result in deposition of silver on the tray, which will decrease the quality of the staining.

• Fixing:

The STERLING Silver Stain Fixative enhances staining for DNA and many proteins. The recommended 5-minute incubation period is optimal for most proteins, but may be adjusted to optimize staining for a specific protein. The proprietary STERLING Fixative is not suitable for long-term storage of gels. If staining is not to be carried out soon after electrophoresis, the gel may be fixed and stored in the standard fixative (5:5:1 Methanol:Water: Glacial Acetic Acid). Gels may be taken directly from this solution into the STERLING fixative to begin the staining procedure.

• Timing:

The entire fixing and staining protocol can be completed in under one (1) hour. The protocols indicate convenient times to make up the stain component solutions. In particular, it is important to confirm that Reagent B is completely dissolved prior to use. This step takes 5-10 minutes and should be verified visually before use.

• Staining:

Agitation of trays during staining and thorough mixing of components A and B prior to placing the gel in the combined staining solutions will give the most consistent and highest quality results. Use a tray of the appropriate size to allow a depth of solution of at least 1cm.

• For Greater Convenience:

- STERLING Reagent A may be diluted and stored as a 1X stock rather than diluting as needed.
- STERLING Reagent B may be dissolved up to 8 hours prior to use.
- STERLING Fixative must be prepared fresh as needed.

TROUBLESHOOTING:

High Background:

1. Accompanied by silver deposition on staining tray: Glassware not sufficiently clean. Wash with Nitric or Chromic Acid prior to use.
2. Accompanied by slower than usual band development: Washes are insufficient. Agitate trays during washes, use non-ionic surfactant at 0.1% in washes to aid in submerging the gel. Use more water per gel.
3. Deionized water is not sufficiently high-quality. Try glass-distilled water if available.

Silver Precipitates when Parts A & B are Combined:

1. Generally, this is caused by residual silver deposits on the glassware used. Acid wash all glassware immediately prior to use to minimize this problem.
2. Reagent B not completely dissolved. Crystals of Reagent B will catalyze silver precipitation.

Spots on Gel:

1. Caused by excessive handling of the gel or by handling with metal instruments or contaminated gloves.

STERLING KIT
Order No. EC-720

Kit (1-3)
Kit (4+)

STORAGE:

STERLING Rapid Silver Stain Kit components are best stored at room temperature (20˚C) and are stable for up to one (1) year.

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